UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

September 21, 2000

SUBJECT: Benomyl/Carbendazim (MBC) Conclusions of an Ad Hoc Carcinogen Assessment Review Committee. D269149. 099101

TO: Demson Fuller
Reregistration Branch 1
Special Review and Reregistration Division (7508C)

FROM: Deborah Smegal, MPH
Health Effects Division (7509C)
Office of Pesticide Programs

Deborah C. Smegal

THROUGH: William Burnam, Chair
Cancer Assessment Review Committee
Health Effects Division (7509C)
Office of Pesticide Programs

On September 6, 2000 an Ad Hoc group of the members of HED's Cancer Assessment Review Committee (CARC) met to evaluate the comments submitted by the Registrant and to determine if a re-evaluation of the carcinogenic potential of benomyl is warranted by the entire CARC. The Registrant submitted their comments on June 14, 2000 in the document titled "Response of E.I. Du Pont De Nemours and Company to Toxicology-Related Issues in the EPA's Draft Reregistration Eligibility Review and Tolerance Reassessment Documents for the Pesticide Benomyl". DuPont Report No. 4413. In addition, information published in the scientific literature that pertained to aneuploidy was discussed as a possible mode of carcinogenic action for benomyl and MBC.

Individuals in Attendance include: Debbie Smegal, Nancy McCarroll, Vicki Dellarco, Dick Hill, Bill Burnam, Mike Ioannou, Esther Rinde, Karl Baetcke, and Alberto Protzel.

Attachments: Review Package

The Registrant provided the following major comments:

(1) The mouse liver tumors are not relevant to humans. Benomyl induced tumors in only one strain of mouse and that strain is susceptible to spontaneous liver neoplasia with a high background incidence. The mechanism of tumor formation in mouse strains with a high tumor incidence is not relevant to humans and is unrelated to aneuploidy. Furthermore, the mechanism of over-expression of the spontaneous liver neoplasms in mice is likely related to the cytochrome P-450 induction and associated cellular proliferation. Benomyl did not induce tumors in a mouse strain with a low background rate of liver tumors or in rats.

(2) EPA should use the margin of exposure approach rather than the linearized multistage (LMS) procedure for assessing cancer risks. The Registrant provides two different opinions to support this statement: (1) "Since the genotoxic effects of benomyl are related to inhibition of microtubule function, the dose response curve for low dose risk is expected to be highly nonlinear." (pg. 16) and, (2) "The mechanism of tumor formation in mouse strains with a high tumor incidence is not relevant to humans and is unrelated to aneuploidy." (p. 17)

(3) If EPA should decide, in error, to evaluate the carcinogenic risks to humans using the LMS model, the current Q_1* value based on MBC tumor data is incorrect for benomyl and should be corrected using a molecular weight conversion factor.

Conclusions:

The ad hoc group concluded that a re-evaluation of the carcinogenicity classification for benomyl/MBC by the Carcinogen Assessment Review Committee (CARC) is not merited at this time because no data have been submitted to establish a mode of action for liver tumor induction in mice. Therefore, the Agency does not agree that the liver tumors are irrelevant to human carcinogenicity. In addition, the available data do not establish aneuploidy or any other nonlinear mode of action as a causal event for the induction of liver tumors. Therefore, the Agency does not agree that a nonlinear mode of action has been demonstrated that would merit a margin of exposure dose response approach.

(1) Regarding the "high background rate" for liver tumors, the ad hoc group noted that in females the concurrent controls had 5% or less incidence. In addition, the relevance to humans of mouse liver tumors cannot be dismissed because data to do otherwise have not been provided. Regarding the claim of cytochrome P-450 induction resulting in overexpression of the spontaneous liver neoplasms in mice, the existing data do not provide substantiation for this claim.

(2) The mutagenicity studies cited in the registrant's comments have been examined. There is little indication that gene mutations or structural chromosome aberrations play a significant role in carcinogenesis of this chemical. Benomyl and structural analogues do produce numerical chromosome aberrations by interfering with tubulin assembly. However, no definitive data linking tubulin binding to liver carcinogenicity were presented by the registrant or are available in the open literature.

(3) Since it was concluded that there is no evidence to support a non-linear mode of action, the Agency will continue to classify both benomyl and MBC as a group C, possible human carcinogen. Regarding the Q_1^* for benomyl, it was concluded that the current Q_1^* of 2.39×10^{-3} $(mg/kg/day)^{-1}$ based on tumor data for MBC should still be used to assess benomyl, as MBC equivalents. Therefore, the estimated exposures (dietary and occupational) should be adjusted based on a ratio of the molecular weights for MBC (190) to benomyl (290) (i.e., multiply the benomyl exposure by a factor of 0.66).

TRADE SECRET

Study Title

RESPONSE OF E. I. DU PONT DE NEMOURS AND COMPANY TO TOXICOLOGY-RELATED ISSUES IN THE EPA'S DRAFT REREGISTRATION ELIGIBILITY REVIEW AND TOLERANCE REASSESSMENT DOCUMENTS FOR THE PESTICIDE BENOMYL

Date Study Completed

June 14, 2000

Submitter

E. I. du Pont de Nemours and Company
DuPont Agricultural Products
Walker's Mill Plaza, Barley Mill
Wilmington, DE 19880-0038

DuPont Report Number

DuPont-4413

COMMENTS ON BENOMYL: HED PRELIMINARY RISK ASSESSMENT FOR THE REGISTRATION ELIGIBILITY DECISION (RED) DOCUMENT

CHEMICAL NO. 0099101. BARCODE: D221850.

APRIL 24, 2000

All relevant comments on the "Toxicology Chapter for Benomyl and Carbendazim.DP Barcode D2640692, Case 819338, Benomyl PC Code 099101, Carbendazim PC Code 128872, March 30, 2000" should be incorporated in the above document (Appendix A, Summary of Toxicological Data for Benomyl and MBC, Page 74).

Section 1.0 EXECUTIVE SUMMARY

Hazard (page 6, paragraph 2)

EPA should state that the mouse liver tumors are not relevant to humans and adjust their classification accordingly. (see discussion below).

Toxicity Endpoints/Dermal Endpoints, (page 7)

Where oral NOAELs are selected, EPA should use a dermal bioavailability factor of 0.2% when the oral NOAEL is from a rat study. (DuPont-4352, see Attachment 1). A further adjustment, based on rat vs. human dermal penetration differences should also be applied, (MRID # 43160001, DuPont Report No. CTL/P/3659), and (MRID # 43160002, DuPont Report No. CTL/P/3833).

Cancer (page 7)

EPA should not use a linearized multistage procedure for assessing cancer risks. Rather, a margin of exposure approach would be more consistent with recent agency cancer risk assessment guidance. Further the Q_1* value is presented without the requisite clause that the true cancer risk is unknown and could be as low as zero. This statement should be made clearly in the Executive summary, (see discussion below).

Section 3.0 HAZARD CHARACTERIZATION

Section 3.1 Hazard Profile Overview

Chronic toxicity and carcinogenicity (page 14)

Benomyl has been classified as a group C carcinogen and the carcinogenic risk to humans was evaluated using the linearized multistage model in order to calculate a Q1*.

DuPont does not agree with EPA's assessment of the carcinogenic potential of benomyl. Benomyl does not interact directly with DNA. EPA's classification of benomyl and MBC is based on weighted evidence of benign and malignant mouse liver tumors. EPA has not given consideration to a subsequent peer review by three pathologists of the slides and data for both the benomyl and the MBC mouse studies. The conclusion of this review was that both compounds produced benign, but not malignant, hepatocellular neoplasms.[1,2]

Benomyl induced liver tumors in only one strain of mouse and that strain is susceptible to spontaneous liver neoplasia with a high background incidence (MRID # 00096514). However, as the Agency notes, MBC did not induce tumors in a strain of mouse with a low spontaneous incidence of liver tumors, (WHO 1993b). Benomyl did not induce tumors in rats. Our conclusion, based also on supplementary mechanistic data, is that the mouse liver tumors are not relevant to humans; and therefore, benomyl should not be classified as an oncogen. Thus EPA's low dose-linear approach to cancer risk assessment is inappropriate.

If EPA should decide, in error, to maintain the group C classification for benomyl, the revisions to the Carcinogen Risk Assessment Guidelines allow for more appropriate methods of assessment.[3] Since the genotoxic effects of benomyl are related to the inhibition of microtubule function, the dose response curve for low dose risk is expected to be highly nonlinear. In fact, a threshold-like dose response has been demonstrated for benomyl and MBC induced aneuploidy [4,5,6,7] Thus, a margin of exposure approach should be applied. Such an approach would be more consistent with the draft revised US EPA carcinogen risk assessment guidelines than applying the default linearized multistage model.

Even if EPA should decide, in error, to evaluate carcinogenic risks to humans based on the LMS model, the current Q_1* value is incorrect. EPA has based the benomyl Q_1* value on an oncogenicity study with MBC. Thus the LMS

[1] Hardisty, J. F. (1990). Oncogenicity studies with benomyl and MBC in mice. Peer review of liver neoplasms. EPL Project No. 129-012.

[2] Frame, S. R., and Van Pelt, C. S. (1990). Oncogenicity studies with benomyl and MBC in mice. Supplemental Peer Review. Supplements to DuPont HLR 70-82, Pathology Report 34-90. (MRID # 41607904)

[3] United States Environmental Protection Agency (1996). Proposed guidelines for carcinogen risk assessment. Fed. Reg. 61(79): 17960-18011.

[4] Bentley, K., Kirkland, D., Murphy, M., and Marshall, R. (2000) Evaluation of thresholds for benomyl- and carbendazim-induced aneuploidy in cultured human lymphocytes using fluorescence in situ hybridization. Mutation Research 464:41-51.

[5] Elhajouji, A., Van Hummelen, P., and Kirsch-Volders, M. (1995) Indications for a threshold of chemically-induced aneuploidy in vitro in human lymphocytes. Environmental and Molecular Mutagenesis 26:292-304.

[6] Elhajouji, A., Tibaldi, F., and Kirsch-Volders, M. (1997) Indication for thresholds of chromosome non-disjunction versus chromosome lagging induced by spindle inhibitors in vitro in human lymphocytes. Mutagenesis 12:133-140.

[7] Marshall, R., Murphy, M., Kirkland, D., and Bentley, K.S. (1996) Fluorescence in situ hybridisation with chromosome-specific centromeric probes: A sensitive method to detect aneuploidy. Mutation Research 372:233-245.

dose-response assessment should be conducted after applying a molecular weight conversion factor to convert MBC to benomyl equivalents.

Mutagenicity (page 16)

The Agency states that benomyl and MBC have marginal mutagenic activity in standard in vitro genotoxicity studies. DuPont would like to point out that it is generally accepted that these substances do not interact with DNA and that they have produced primarily negative results for gene mutation, chromosome aberrations, and DNA damage and repair *in vitro* and *in vivo*. Although spurious positive results have been produced in some *in vitro* gene mutation or SCE studies with MBC, these results were attributable to trace quantities of mutagenic phenazine process impurities (mentioned by EPA in Table 14 in the Toxicology Chapter for Benomyl and Carbendazim (DP Barcode D2640692). Studies with highly purified MBC (~100%) have produced negative results.

In addition to the studies summarized by the Agency, recent reports in the published literature have clearly demonstrated that the induction of aneupolidy by these mitotic spindle inhibitors exhibit a characteristic dose-response pattern which includes a threshold.[4,5,6,7] The shape of the dose-response curve is similar to that of a ligand-receptor mediated mechanism of toxicity, in this case, the binding to tubulin and the inhibition of microtubule function. Only when the critical threshold concentration is reached and a sufficient number of spindle fibers are affected is aneuploidy induced. We suggest that these studies also be noted in the Agency's review of the available mutagenicity studies for these substances.

DuPont disagrees with the Agency's statement that the mutagencity data support the evidence of hepatocellular tumors in mice. As pointed out in the comments above and also by the Agency on page 14 of the Toxicology Chapter for Benomyl and Carbendazim (DP Barcode D2640692), hepatocellular carcinomas were observed only in strains known to have a high background incidence of liver tumors. In contrast, no liver tumors were produced by MBC in a mouse strain with a low background incidence and no tumors were observed in rat studies conducted with either compound. (WHO, 1983b). We believe that these substances have been incorrectly classified as carcinogens and that the mechanism of tumor formation in these sensitive mouse strains is not relevant to humans and is unrelated to aneuploidy.

Metabolism and Pharmacokinetics (page 16)

EPA has summarized the metabolism and pharmacokinetics of benomyl based on an acceptable rat study with MBC. However, DuPont has recently completed an extensive set of pharmacokinetic studies in rat, rabbit, dog, and cynomolgus monkey. These studies provide new information on the plasma metabolite profile and kinetics and should be incorporated into the risk assessment, (see Attachment 1).

Dermal Absorption (page 17)

EPA has applied a dermal absorption factor of 3.5% when deriving a dermal exposure RfD based on oral dose data. The new metabolism and pharmacokinetics data referred to above demonstrate that dermal bioavailability is 0.2-0.4% (DuPont-4352, see item 1 on Attachment 1). See comments on the Re-evaluation Report of the Hazard Identification Assessment Review Committee EPA HIARC document.

Section 3.3.1 Non-Cancer Endpoints (page 20)

Benomyl

In the text and in footnote (b) of Table 3, the degree of protection afforded by the dermal NOAEL is understated. The margin of protection should be calculated by adjusting the 30 mg/kg/day value by 0.002, the fractional bioavailability for rats determined in the recent dermal metabolism and pharmacokinetics studies.

Section 3.3.2. Classification of Carcinogenic Potential (page 22)

EPA has classified benomyl and MBC as group C carcinogens. DuPont disagrees with this classification and believes that the risk assessment for potential carcinogenic effects should be based on a margin of exposure approach rather than a linearized multistage procedure, (see comments in Section 3.1).

EPA should not evaluate cancer risk using the linearized multistage model. As stated in our comments above in section 3.1, the liver tumors observed in mice, the only species to develop tumors in response to benomyl or MBC administration, are not relevant to human health risk. Furthermore, the mechanism of over-expression of these spontaneous neoplasms is likely related to the cytochrome P-450 induction and associated cellular proliferation. Thus a low dose linear method of risk extrapolation is entirely inappropriate for this mechanism.

Finally, EPA has calculated a unit risk value for benomyl based on linearized multistage modeling of the MBC mouse liver tumor data. The Q_1^* for MBC has been applied, in error, directly to benomyl without making the appropriate molecular weight conversion. Thus, if EPA continues to use a Q_1^* approach, the value reported in Table 3 must be specified as applying to MBC and an additional value should be calculated for benomyl. This change should be carried through in all subsequent risk calculations.

EPA presents only the upper bound cancer potency value without stating explicitly, as required by EPA Risk Assessment Guidelines, that these values are estimates, the true value of the risk is unknown, and may be as low as zero.

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E. Rinde

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 24 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Benomyl/Carbendazim (MBC) Conclusions of an Ad Hoc Carcinogenicity Peer Review Meeting (DP Barcode reference #: D195377)

FROM: Melba S. Morrow, D.V.M. 8/7/95
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Susan Cerrelli/Linda Propst, PM 73
Reregistration Division (H7508C)

THRU: Joycelyn E. Stewart, Ph.D. 8/7/95 8/21/95
Head, Section II
Toxicology Branch I
Health Effects Division (H7509C)

CONCLUSIONS:

On Wednesday, July 26,1995, an ad hoc Health Effects Division (HED) Carcinogenicity Peer Review Group convened to discuss the impact that a re-evaluation of mouse liver slides would have on the carcinogenicity classification of both Benomyl and MBC. The meeting was attended by Karl Baetcke, Bill Burnam, Marion Copley, Kerry Dearfield, Esther Rinde (all members of the HED Carcinogenicity Peer Review Committee), Kathy Martin (RCAB), Joycelyn Stewart and Melba Morrow (TBI).

The ad hoc group agreed with the conclusions in the attached memo (Morrow to Cerrelli, dated January 5, 1995) and DER. It was concluded that with regard to the carcinogenic potential of the compounds in question, the re-review of the liver slides do not demonstrate that Benomyl and MBC are not carcinogens. For Benomyl, re-review of the data demonstrate an increase of hepatocellular adenomas at the low and mid doses in male CD-1 mice and at the high doses in female mice. High dose male mice did not show any increase in tumor incidence (either adenomas or carcinomas, but did show a significant increase in the incidence of foci of cellular alteration which was significant by pairwise comparison at $p < 0.05$. The foci of cellular alteration were also increased in mid dose males, but not significantly so. In

II. POLICY AND RATIONALE

The Agency believes that a procedure for obtaining consensus in pathology re-reads will improve the quality of decision-making in classifying pesticide chemicals having carcinogenic potential. The Agency has determined that unless the re-reads have been conducted using a Peer Review procedure, the Agency will base its evaluations upon the original readings.

The following will be required:

For any target tissue which is being re-evaluated, all slides containing that tissue in all dose groups, as well as the controls, must be re-read by the peer review pathologist. This is to include slides previously classified by the study pathologist as within normal limits, in addition to those having tumors, hyperplasia, hypertrophy, foci of cellular alteration or other non-neoplastic lesions.

The pathology reports from both the study and peer review pathologist and the original slides are to be submitted to a Pathology Working Group (PWG) similar to that described in the NTP Technical Reports under the section: "Clinical Examinations and Pathology." The PWG will review, as a minimum, all slides about which there were significantly differing diagnoses between the study and peer review pathologists.

Finally, the Agency should be provided with a detailed pathology report, which presents the PWG findings and includes the original diagnosis and the new diagnosis for each slide read, and a comment column to note any discrepancies, missing slides, etc.

The Agency also is considering including the requirement for review by a PWG for all original submissions in the future. This present Notice deals only with re-reads.

III. EFFECTIVE DATE

This policy notice is effective immediately. If you have questions, contact Esther Rinde at (703) 305-7492.

Penelope A. Fenner-Crisp,
Deputy Director (Acting)
Office of Pesticide Programs

[1] From: DCOPP3-POADMIN 8/30/94 10:28AM (4803 bytes: 103 ln)
Subject: PR Notice 94-5 (8/24/94)
---------- Message Contents ----------

August 24, 1994

PESTICIDE REGULATION (PR) NOTICE 94-5
NOTICE TO REGISTRANTS OF PESTICIDE PRODUCTS

ATTENTION: Persons Responsible For Registration of Pesticide Products

SUBJECT: Requests for Re-considerations of Carcinogenicity Peer Review Decisions Based on Changes in Pathology Diagnoses.

This notice sets forth a procedure to be followed for submission of pathology re-reads to the Agency.

I. BACKGROUND

From time to time the Office of Pesticide Programs receives requests for re-consideration of Peer Review decisions based on re-evaluations of the pathology readings. These re-evaluations reflect voluntary activity on the part of the registrants, and are not the result of a requirement imposed by the Agency. The Agency is then asked to disregard the original readings and base its evaluation on the most recent ones. As a result the Agency may have two (or at times even more) pathological diagnoses for the same study.

Since this situation is occurring more and more frequently, the Agency is instituting a procedural requirement for any voluntary submissions of revised pathology diagnoses. This procedure will require a comprehensive peer review process, similar to the one used by the National Toxicology Program (NTP).

The National Toxicology Program (NTP) has a protocol for quality assurance in pathology, involving a quality assessment (peer review) pathologist and a Pathology Working Group (PWG) which is used to resolve differences in diagnoses between the laboratory (study) pathologist and the peer review pathologist. The PWG consists of a chair, the peer review pathologist and other pathologists (to include the study pathologist), all of whom are experienced in rodent toxicologic pathology. This group examines the tissues without knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differs from the opinion of the study pathologist, the diagnosis is changed. Thus, the final diagnoses represent a consensus of study, peer review, and consultant pathologists on the PWG. This procedure is described in the NTP Technical Reports under the section: "Clinical Examinations and Pathology." EPA believes that the use of a PWG, similar to one used by NTP, should be part of every pathology re-evaluation.

4/7/89

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FILE COPY

APR 7 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Third Peer Review of Benomyl/MBC

FROM: John A. Quest, Ph.D., Head JAQ 2/14/89
Science Support Staff
Science Analysis and Coordination Branch
Health Effects Division (TS-769C)

TO: Jane Mitchell, PM Team 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

The Health Effects Division Peer Review Committee met on January 25, 1989 to discuss whether or not the tumor data on Benomyl/MBC necessitated a quantification of oncogenic risk.

A. Individuals in Attendance

1. Peer Review Committee: (Signature indicates concurrence with peer review unless otherwise stated.)

Robert Beliles: Robert Beliles

William Burnam: William Burnam

Marion P. Copley: Marion P. Copley

Bernice Fisher: Bernice Fisher

Marcia van Gemert: Marcia van Gemert

Judith W. Hauswirth: Judith W. Hauswirth

John A. Quest: John A. Quest

William Sette:

2. Peer Review Committee Members in Absentia:
(Committee members who were not able to attend the discussion; signature indicates concurrence with the overall conclusions of the committee).

Reto Engler: Reto Engler

Richard N. Hill: —

Diane Beal: —

Kerry Dearfield: Kerry Dearfield

Lynnard Slaughter: L. Slaughter

Esther Rinde: Esther Rinde

Richard Levy: Richard A Levy

3. Interested Observers:

Albin Kocialski: A. Kocialski

Phil Hundemann: _____

B. Material Reviewed:

This material available for review by the Committee was a package prepared by Dr. Copley containing information on most of the major scientific and regulatory activities conducted by the OPP over the past several years.

C. Background

Background information on Benomyl/MBC is comprehensively provided in Dr. Copley's memorandum of January 20, 1989 (attached). In brief, at the Peer Review Committee meeting of January 7, 1986, it was determined that Benomyl/MBC met some of the criteria for both the B2 and C categories of carcinogen classification. In support of a B2 category classification, both Benomyl and MBC produced an increased incidence of malignant or combined malignant and benign tumors of the liver. In the case of MBC, tumors were produced in multiple strains of mice (closely related CD-1 and Swiss SPF strains) and in multiple experiments. Furthermore, MBC produced an unusual type of hepatocellular tumor (hepatoblastoma) in male Swiss SPF mice. In support of a C category classification, it was noted that: 1) the oncogenic responses observed with Benomyl and MBC were confined solely to the mouse liver, even with repeated experiments; 2) the liver tumors produced by Benomyl and MBC were observed in two related strains of mice (CD-1 and Swiss

SPF) known to have high background incidence rates of liver tumors whereas no liver tumors were produced by MBC in another strain of mice [HOE NMRKf (SPF 71)] known to have a low background incidence rate of liver tumors; and 3) Benomyl and MBC produced weak mutagenic effects consistent with spindle poison activity rather than gene mutation or DNA repair activity.

Based on the above information, the Peer Review Committee decided that there was insufficient evidence for the B2 category and classified Benomyl/MBC as a Category C oncogen. Although there was some discussion by the Committee of possible quantification of risk, a formal decision about whether or not to quantify was not made. A similar situation prevailed at an SAP meeting on Benomyl/MBC held May 21, 1986. It should be noted that at that time, HED had calculated interim estimates of cancer potency for both Benomyl ($Q1^* = 5.9 \times 10^{-3}$; human risk) and MBC ($Q1^* = 3.9 \times 10^{-3}$; human risk) using tumor information from the female mouse portion of an MBC study where the incidence of liver tumor bearing animals (adenomas, carcinomas, and hepatoblastomas was 1/79 at 0 ppm, 9/78 at 500 ppm, 21/80 at 1500 ppm, and 15/78 at 7500 ppm. To resolve the outstanding issue of whether the group C categorization of Benomyl/MBC is appropriate for quantification of risk using the Q1*, the Registration Division requested that the present Peer Review Committee be convened.

D. Conclusion of the Peer Review Committee on Risk Quantification

The Committee determined that quantification of risk was warranted for Benomyl/MBC in view of the above described biological data supportive of the category B2 classification. In particular, this data included the occurrence of a mostly malignant hepatocellular tumor response with MBC in two strains of mice (and with Benomyl in one strain of mouse), the fact that the malignant response was generally seen in both sexes of mice, and the presence of the unusually occurring and malignant hepatoblastomas with MBC in male SPF Swiss mice. In addition, mutagenicity information was provided by Dr. Dearfield indicating that the aneuploidy (i.e., loss of chromosome material) known to be produced by Benomyl could theoretically result in a loss of tumor suppressor genes and a potential oncogenic effect (see Cancer Research 48:1623-1632, 1988).

The assignment of a Q1* value for human risk to Benomyl/MBC was temporarily deferred until a brief review of the incidence data for MBC-induced liver tumors in female mice is conducted to check for numerical accuracy of numerator and denominator values. In all probability, the

Q1* value cited above in this document for MBC will be employed for MBC and Benomyl.

Other Deliberations of the Committee

The Committee also briefly considered whether a quantitative risk assessment should be performed on Thiophanate Methyl, another pesticide that, like Benomyl, is metabolized to MBC in both animals and plants. It was decided that the Q1* value derived for MBC from Benomyl metabolism could now be used to characterize the Q1* for MBC derived from Thiophanate-Methyl metabolism, provided that the latter agent results in MBC residues on plants. This issue can be considered further in the future when Thiophanate Methyl per se is peer reviewed. At present, a chronic mouse study on Thiophanate methyl is outstanding and the Committee could not comment further on this parent compound.

In view of the Agency's issue paper on mouse liver tumors and the recent workshop held in Virginia Beach, Virginia, both of which discussed the relevance of these tumors to humans, the Committee considered that the need for quantitative risk assessment on Benomyl/MBC could be modified. Further information on Benomyl/MBC that could influence this decision would include data on comparative metabolism, peroxisome proliferation, hepatic microsomal drug metabolism, and hepatocytotoxicity in mice. The Committee will schedule a separate meeting to discuss these generic issues.

Attachment

TOXICOLOGY SUMMARY FOR THE THIRD PEER REVIEW OF BENOMYL AND MBC

Data Evaluation Report for the
Third Meeting of the
Peer Review Committee for Benomyl and MBC

Submitted by Marion P. Copley, D.V.M., Sect.2, Tox. Br.1, HED
Through Judith Hauswirth, Ph.D., Branch Chief
Toxicology Branch 1 (IRS), Hazard Evaluation Division

completed January 19, 1989

TOXICOLOGY SUMMARY FOR THE THIRD PEER REVIEW OF BENOMYL AND MBC

Data Evaluation Report for the
Third Meeting of the
Peer Review Committee for Benomyl and MBC

Data Evaluation Report for the Third Meeting of the
Peer Review Committee for Benomyl and MBC

Submitted by Marion P. Copley, D.V.M., Sect.2, Tox. Br.1, HED
Through Judith Hauswirth, Ph.D., Branch Chief
Toxicology Branch 1 (IRS), Hazard Evaluation Division

1. Issues

The Hazard Evaluation Division (HED) Peer Review Committee (formerly the Toxicology Branch (TB) Peer Review Committee) is requested to:

a) reevaluate whether Benomyl and MBC should be evaluated using the multistage model of risk quantification. This should take into consideration that this Committee already classified Benomyl and MBC as C oncogens based on liver tumors.

b) If a Q_1* is deemed appropriate, to determine whether the previous calculations are adequate or whether they should be redone.

2. Background

a) Benomyl produces liver tumors, both hepatocellular adenomas and hepatocellular carcinomas in two closely related strains of mice (males and females) but not in an unrelated strain of mice or in rats.

Benomyl and MBC were discussed by the Peer Review Committee first on 10/3/85. At that time additional information was requested from the reviewer. No Peer Review Document resulted from that preliminary meeting. On 12/19/85 the Committee reconvened and following review of tumor data, metabolism and structure-activity information, historical control information, mutagenicity data and a listing of one-liner material, classified both fungicides as Category C (possible human) carcinogens.

Although it was discussed at some length, the Committee did not establish whether this compound was suitable for risk quantification by the standard procedures.

b) Benomyl has undergone a complete Special Review cycle. The result of the PD4 (10/1/82) was to regulate exposure by requiring dust masks.

A risk quantification was conducted for the PD4 with the Q1* of 2.065×10^{-3} $(mg/kg/day)^{-1}$. This was based on a benomyl chronic/oncogenicity study that has since been core-graded as supplementary for oncogenicity. Since that time a new value for the human Q1* was calculated : 3.9×10^{-3} $(mg/kg/day)^{-1}$ (see appendix 4 for statistical memos). This used data from

an MBC study which was core-graded minimum for oncogenicity.

NOTE: As stated in the PD4, benomyl rapidly hydrolyses to MBC in an aqueous environment. MBC also appears to be the initial metabolite in mammalian systems. It has similar or increased toxicity, both acute and chronic, to benomyl. For these reasons MBC data has been used to confirm and supplement benomyl data where applicable.

c) Benomyl was presented to the Scientific Advisory Panel in 5/21/86. They agreed with the classification of Benomyl and MBC as class C (possible human) carcinogens. No comment was given to the question of when to quantify using the multistage model. However the panel stated that,

> "... Benomyl and its major metabolite ... MBC produce tumors in livers of two genetically related strains of mice. It does not produce tumors in a genetically unrelated mouse strain nor does it produce tumors in a two-year rat study. Both benomyl and MBC produce weak mutagenic effects consistent with spindle poison activity rather than gene damage and DNA repair activity. In view of these species differences in oncogenic activity and lack of evidence of any direct action on DNA, there are reasonable grounds for doubt that benomyl and its major metabolite MBC are human oncogens. The Panel believes that the classification C seems appropriate."

d) There have been two MBC studies reviewed since the previous peer review. They were discussed and World Health Organization summaries of these studies were included with the previous peer review. Attached in Appendix 5 are completed DERs for:

1) Repeated-dose (24-month) feeding study for determination of the cancerogenic effect of HOE 17411 O F AT204 (carbendazim) in mice. (NMRKf(SPF71) strain)

and

2) Carcinogenicity study with Carbendazim in mice. (Swiss random strain)

3. Summary Weight-of-the-Evidence

Category C oncogen (possible human oncogen) for Benomyl and MBC

1. Tumors in one specie (mouse)
2. Tumors in two strains of mouse (CD-1 and Swiss random)
   a. Tumors in two sexes (of above studies)
   b. Both benign and malignant hepatocellular tumors
   c. Genetically related - both are outbred derivitaves of the Swiss strain
   d. Both strains have high historical control values for liver tumors in male mice
   e. Tumors limited to one organ (liver)
   f. Tumors only at end of study
   g. Tumors primarily only at high doses
   h. No evidence for metastases or invasion
   i. No evidence for decreased time to occurrance of tumors.
3. Tumors not in one (genetically unrelated) strain
   a. NMRKf strain;
   b. Low historical control values for liver tumors.
   c. Evidence for hepatotoxicity is present
4. Mutagenicity - weak
   a. Genotoxicity - equivocal: DNA repair, gene mutation
   b. Cytotoxicity - Spindle inhibition
5. Teratogenic (microphthalmia in mice)

3/31/86

FILE COPY

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 31 1986

MEMORANDUM

1st + 2nd

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Benomyl and MBC

FROM: for John A. Quest, Ph.D.
Team Leader, Scientific Mission Support Staff
Toxicololgy Branch/HED (TS-769)

TO: Henry Jacoby
Product Manager #21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

The Toxicology Branch Peer Review Committee met on January 7, 1986 to discuss and evaluate the data base on Benomyl and its primary metabolite, MBC,, with particular reference to the oncogenic potential of the chemical. A preliminary meeting was held on October 3, 1985 on Benomyl to determine the information that would be required to hold an in-depth discussion on this compound.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber

William Burnam

Anne Barton

Reto Engler

R. Bruce Jaeger (see attached)

Bertram Litt

John A. Quest

2. Reviewers: (Non-panel members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Marion Copley

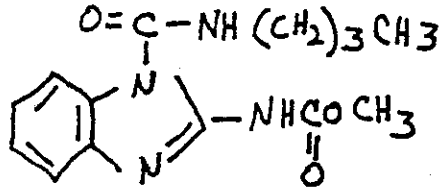
Jane E. Harris

B. Material Reviewed:

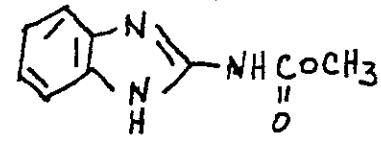
The material available for review consisted of a comprehensive summary of toxicology information on Benomyl (Copley/Harris memorandum dated 12/19/85), including tumor data on Benomyl and its major metabolite MBC, metabolism and structure-activity information, historical control information, mutagenicity data, and a listing of one-liner material on the Benomyl/MBC data base. A copy of the information reviewed is appended to this panel report.

C. Background Information:

Benomyl is a benzimidazole carbamate compound (methyl-1(Butylcarbamoyl)-2-benzimidazolecarbamate) that is metabolized under aqueous conditions both in vivo and in vitro to its major metabolite MBC (methyl 2-benzimidazole carbamate). Both compounds are systemic fungicides and both are associated with hepatocellular tumors in certain strains of mice but not in rats.



BENOMYL



MBC

The Review Committee evaluated oncogenicity data on these chemicals from 4 studies performed in mice and from 2 studies performed in rats. The oncogenicity data are summarized below:

D. Evaluation of Oncogenicity Evidence for Benomyl and MBC:

1. Mouse Oncogenicity Study of Benomyl:

Haskell Laboratory administered Benomyl in the diet to groups of 80 male and 80 female Charles River CD-1 mice at concentrations of 0, 500, 1500 or 7500/5000 ppm for 2 years. The high dose of 7500 ppm was reduced to 5000 ppm at 37 weeks in males and females due to weight loss. The following incidence patterns of tumors suggestive of a compound related effect were observed.

Tumor Site and Type	Sex	Dose (ppm) 0	500	1500	7500/5000
Lung:					
Alveologenic carcinoma	M	13/79(16%)	24/79(30%)*	23/79(29%)*	16/80(20%)
	F	16/77(21%)	7/79(9%)	4/78(5%)	6/74(8%)
Liver:					
Adenoma	M	9/77(12%)	9/80(11%)	11/79(14%)	10/80(12%)
Carcinoma	M	16/77(21%)	26/80(32%)*	41/79(52%)*	17/80(21%)
Combined	M	25/77(32%)	35/80(44%)*	52/79(66%)*	27/80(34%)
Adenoma	F	2/77(2.5%)	2/80(2%)	7/79(9%)	7/77(9%)
Carcinoma	F	2/77(2.5%)	7/80(9%)*	6/79(7%)	14/77(18%)*
Combined	F	4/77(5%)	9/80(11%)	13/79(16%)*	21/77(27%)*

*= $p<0.05$ compared to controls

Pulmonary carcinomas were significantly elevated in male mice (low and mid doses). The effect did not appear to be compound related however, since a dose-response effect was not observed in the Cochran-Armitage test for trend; the observed incidences were within the range of historical control rates for this tumor in other studies conducted at Haskell Labs (i.e., 16% to 36%); and the MBC metabolite did not produce an increase in pulmonary tumors in other studies performed in CD-1 mice.

Hepatocellular carcinomas were significantly elevated in male (low and mid doses) and female (low and high doses) mice. In addition, adenomas and carcinomas combined were significantly elevated in males (low and mid doses) and females (mid and high doses). The tumorigenic responses appeared to be compound related; e.g., they occurred with significant positive trends, and the elevated incidences exceeded historical rates for these tumor responses in 2 other studies (an "unnamed" study, and the MBC study cited below under No. 2) conducted at the registrant's laboratory (see Copley/Harris memorandum of 12/19/85, page 10 for data). Furthermore, similar liver tumorigenic responses were produced by the MBC metabolite in other studies performed in CD-1 mice (see below). The oncogenic responses that were produced by Benomyl in treated mice were not accompanied by increased incidences of hepatocellular adenomas or hyperplasia.

The highest dose of benomyl tested in male mice in this study probably exceeded a MTD level. This dose in males produced a decreased weight gain (approximately -9%), hepatocellular toxicity (e.g., foci of cellular alteration, cytomegaly, and foci of degeneration), and degenerative changes in the testes (e.g., atrophy, seminiferous tubule degeneration, and interstitial cell hyperplasia) and in the epididymis (aspermia). This dose did not produce liver tumors in males, possibly because of the hepatocellular toxic changes that were observed (e.g., the observed liver toxicity may have altered the ability of benomyl to be metabolized to MBC). The low and mid dose levels of benomyl did produce liver tumors in males, but these doses were not associated with any other toxic effects and thus did not approximate a MTD level.

The highest dose of benomyl tested in females probably approximated a MTD level as evidenced by findings of decreased weight gain (approximately -9%), elevated liver weights, reduced kidney weight, and spleen hemosiderosis. This dose in females did produce liver tumors, as did lower doses of the compound. Benomyl did not produce the exaggerated liver toxic changes in female mice that were observed in male mice.

2. Mouse Oncogenicity Study of MBC:

Haskell Laboratory administered MBC in the diet to groups of 80 male and 80 female Charles River CD-1 mice at concentrations of 0, 500, 1500, 7500 (females) or 7500/3750 (males) ppm for 2 years. The high dose of 7500 ppm was reduced to 3750 ppm at 66 weeks in males due to increased mortality, and all males were ultimately sacrificed at 73 weeks. The following incidence pattern of liver tumors was observed.

Liver Tumor Type	Sex	Dose (ppm) 0	500	1500	7500**
Adenoma	M	11/80(14%)	15/80(19%)	14/80(17%)	3/80(4%)
Carcinoma	M	2/80(2%)	5/80(6%)	9/80(11%)*	0/80(0%)
Combined	M	13/80(16%)	20/80(25%)	23/80(28%)*	3/80(4%)
Adenoma	F	0/79(0%)	5/78(6%)*	5/80(6%)*	3/78(4%)
Carcinoma	F	1/79(1%)	4/78(5%)	15/80(18%)*	12/78(15%)*
Hepatoblastoma	F	0/79(0%)	0/78(0%)	1/80(1%)	0/79(0%)
Total	F	1/79(1%)	9/78(11%)*	21/80(26%)*	15/78(19%)*

* = $p < 0.05$ compared to controls
** = Reduced to 3750 ppm in males at 66 weeks.

Hepatocellular carcinomas, and adenomas and carcinomas combined, were significantly elevated in male mice (mid dose level); no increase in adenomas occurred in males. The lack of oncogenic response in high dose males is likely to be explained by their early deaths and sacrifice at 73 weeks. In female mice there were significant increases in adenomas (low and mid doses), carcinomas (mid and high doses), and adenomas and carcinomas (all 3 dose level tested). The Committee noted that this profile of liver tumors resembled that described above for benomyl in CD-1 mice. No increased incidence of liver hyperplasia occurred in treated mice. A comparison of the MBC liver tumor data with historical control data from 2 other studies conducted at Haskell Laboratory (the "unnamed" study and the benomyl mouse study in CD-1 mice; see Copley/Harris memorandum of 12/19/85, page 10) indicated that only the carcinomas (mid and high dose levels) and the adenomas/carcinomas combined (all 3 dose levels tested) in female mice exceeded the control response rates in the other studies.

The high dose level of MBC tested in male mice clearly exceeded a MTD level because of excessive mortality. The mid dose level appeared to approximate a MTD level. Both of these doses in males caused reduced weight gain, hepatocellular toxicity (e.g., pigmented macrophages, hypertrophy, and centrilobular necrosis), renal tubular pigmentation, thymic lymphoid depletion, and sperm stasis. The changes however were more severe at the high dose level.

The highest dose of benomyl tested in females appeared to approach but did not exceed the MTD level. This dose caused increased liver weight and foci of eosinophilic hepatocellular alteration, renal tubular pigmentation, and thymic lymphoid depletion.

3. Mouse Oncogenicity Study of Carbendazim (99% MBC):

In a study performed by the Central Institute for Nutrition and Food Research, TNO, and reviewed in summary form by the WHO (see Copley/Harris memorandum of 12/19/85, page 7), MBC was administered in the diet to groups of 100 male and 100 female SPF Swiss mice at concentrations of 0, 150, 300 or 1000/5000 ppm for 80 weeks. The 1000 ppm concentration was increased to 5000 ppm in males and females at week 8. Data were presented in summary form only. The following incidence pattern of liver tumors was observed (Note: In this study the term "neoplastic nodule" was used in place of the term "adenoma"; the term "hepatoblastoma" refers to a more uncommon and malignant type of liver tumor than hepatocellular carcinoma).

Liver Tumor Type	Sex	Dose (ppm) 0	150	300	1000/5000
Neoplastic Nodule	M	9/100(9%)	7/98(7%)	14/100(14%)	16/100(16%
Carcinoma	M	1/100(1%)	1/98(1%)	9/100(2%)	3/100(3%)
Hepatoblastoma	M	0/100(0%)	1/98(1%)	1/100(1%)	7/100(7%)
Total	M	10/100(10%)	8/98(8%)	16/100(16%)	17/100(17%
Neoplastic Nodule	F	0/97(0%)	1/99(1%)	1/98(1%)	9/97(9%)*
Carcinoma	F	1/97(1%)	0/99(0%)	0/98(0%)	0/97(0%)
Hepatoblastoma	F	0/97(0%)	0/99(0%)	0/98(0%)	0/97(0%)
Total	F	1/97(1%)	1/99(1%)	1/98(1%)	9/97(9%)

*= P<0.01 compared to controls, Exact test.

Hepatoblastomas were significantly elevated in male mice (high dose level), and neoplastic nodules (i.e., adenomas) were significantly elevated in female mice (high dose level). The Committee noted that the Swiss SPF strain of mouse used in this study is similar to the CD-1 strain of mouse in which benomyl and MBC were tested; both strains are Swiss derived and tend to exhibit a high background incidence of liver tumors in male mice.

Based on the summary information available for this study, the highest dose level of MBC tested did not appear to exceed a MTD level. The HDT caused increased relative liver weights and clear cell and/or mixed hepatic cell foci in males and females.

4. Mouse Oncogenicity Study of Carbendazim (MBC):

In another study reviewed by the WHO (see Copley/Harris memorandum of 12/19/85, page 8), MBC was administered in the diet to groups of 100 male and 100 female HOE NMRKf (SPF 71) mice at concentrations of 0, 50, 150, 300 or 1000/5000 ppm for 22 months. The 1000 ppm concentration was increased to 5000 ppm at week 8. No evidence of an oncogenic response in the liver or at any other site was observed. The Committee noted that the NMRKf strain of mouse, in contrast to Charles River CD-1 and Swiss SPF mice, normally exhibits a low background incidence of liver tumors.

The highest dose of MBC tested in this study appeared to be close to a MTD level as indicated by findings of liver toxicity in both male and female mice (e.g., liver cell hypertrophy, clear cell foci, liver cells in mitosis, pigmented Kupffer cells, enlarged cell nuclei, and multiple cell necrosis).

5. Rat Oncogenicity Studies of Benomyl and MBC:

Benomyl was studied in a 2-year dietary study (0, 100, 500 or 2500 ppm) in Charles River CD rats; the highest concentration was a systemic NOEL and no oncogenic effects occurred. MBC was also studied in a 2 year dietary study (0, 100, 500, 2500/10,000 or 5000 ppm) in Charles River CD rats; on oncogenic effects occurred. In this study, the highest dose level was a MTD level as evidence by findings of weight loss in males and females (10%-20% less than controls) and hepatic pericholangitis. Both of the above studies were performed by Haskell Laboratory.

E. Additional Toxicology Data on Benomyl and MBC:

1. Metabolism:

Limited studies conducted in mice indicate that benomyl is primarily metabolized to MBC, which in turn is converted to 2-aminobenzamidole (2-AB) and also to 5-OH-MBC and 5-OH-2-AB. The latter 2 metabolites undergo sulfate and glucuronide conjugation. Elimination of metabolites occurs rapidly in urine and feces (e.g., 94% of an orally administered radiolabelled dose was excreted in 96 hours in mice as the metabolites, with no parent compound detected). No unusual localization of benomyl or its metabolites has been found in animal tissues.

2. Teratology:

Benomyl has been demonstrated to be teratogenic in several oral (gavage) studies conducted in both Wistar and Charles River CD rats at doses ranging from 62.5 to 125 mg/kg/day. The most common abnormality in these studies was microphthalmia. In most of these studies, fetotoxic and embryotoxic effects were also observed at similar or greater dose levels. Benomyl was also reported to be teratogenic in one study in Charles River CD-1 mice at oral (gavage) doses of 100 mg/kg or more. The major anomalies noted were cleft palate, supernumerary ribs, and subnormal verterbral centrum (no compound-related microphthalmia was reported).

3. Mutagenicity:

Data provided in the Position Document 4 on Benomyl and MBC indicated that both compounds are spindle poisons. For example, nondisjunction was reported in A. nidulans and many other test systems with both agents. The compounds

also produced positive effects in tests to assess structural chromosome abberations which were consistent with a spindle effect; e.g., benomyl was weakly positive for sister chromatid exchange in vitro in Chinese hamster ovary cells with and without activation, and both benomyl and MBC caused increased incidences of micronuclei in polychromatic erythrocytes in mice bone marrow. In other studies performed to assess gene mutations, equivocal results were obtained. That is, MBC was weakly positive in one mouse lymphoma test (L5178Y $TK^{+}/-$) but was negative in a second test, Benomyl and MBC produced both positive and negative results in different Ames tests, and both compounds produced negative results in Chinese hamster ovary cells (HGPRT). Finally, negative results were obtained for DNA repair with Benomyl and MBC in several studies in primary mouse and rat hepatocyte cultures. The Peer Review Committee was of the opinion that these results, when taken together, indicated that both Benomyl and MBC have weak mutagenic activity that is primarily attributable to adverse effects on the cellular spindle apparatus. The pattern of results observed did not appear to correlate with heritable desease or oncogenic effects, but may relate to the teratogenic effects observed with Benomyls.

4. Structure-Activity Correlations:

Both Benomyl and MBC bear a close structural resemblance to several other benzimidazole compounds that are suspect oncogens (e.g., fenbendazole and albendazole). The potential oncogenic effects of these compounds are currenly under review by the Center for Veterinary Medicine, Food and Drug Administration and were recently discussed in a Congressional Subcommittee Hearing (reference: Human Food Safety and the Regulation of Animal Drugs; 27th Report by the Committee on Government Operations, December 31, 1985. Union Calendar, No. 274. Intergovernmental Relations and Human Resources Subcommittee. Ted Weiss, New York, Chairman; pp. 1-115). In the case of fenbendazole, a high incidence of liver nodular hyperplasia and low incidences of liver neoplastic nodules, adenomas and carcinomas were observed in rats. In the case of albendazole, histiocytic sarcomas were observed in rats and uterine polyps were observed in rats and mice. The Committee was aware that final decisions regarding the classification of these chemicals as oncogens had not yet been made by the FDA.

F. Weight of Evidence Considerations:

The committee considered the following facts regarding toxicology data on Benomyl and MBC to be of importance in a weight of the evidence determination of oncogenic potential.

1. Benomyl (methyl-1(butylcarbamoyl)-2-benzimidazole carbamate) and MBC (methyl-2-benzimidazole carbamate) are structurally related compounds. Pharmacokinetic studies in mice have demonstrated that Benomyl is rapidly metabolized to MBC in vivo, and that MBC is the primary metabolite of Benomyl. The toxicity of Benomyl may be primarily due to the formation of the MBC metabolite.

2. Both Benomyl and MBC produced significantly elevated incidences of liver tumors (e.g., carcinomas, and carcinomas and adenomas combined) in male and female Charles River CD-1 mice, a non-inbred strain of Swiss mouse known to exhibit a high background incidence of liver tumors in males. (see Sher; Toxicol. Appl. Pharmacol. 30: 337, 1974; and historical control data in Copley/Harris memo of 12/19/85, page 10). The tumors were observed at similar dose levels for Benomyl and MBC, and were also similar in both incidence and type. No hepatocellular hyperplasia was observed in Charles River CD-1 mice exposed to either chemical, but there were increases in foci of cellular alteration.

3. MBC also produced a significantly elevated incidence of liver tumors (i.e., hepatoblastoma - a more uncommon and malignant tumor than hepatocellular carcinoma) in male Swiss SPF mice and a significantly elevated incidence of liver neoplastic nodules (i.e., adenomas) in female mice of the same strain. The CD-1 strain of mouse is similar to the Swiss SPF strain of mice in that it is Swiss-derived and also exhibits a high background incidence of liver tumors in males (Sher, 1974).

4. The tumorigenic responses observed with both Benomyl and MBC in Charles River CD-1 mice (e.g., carcinomas, and carcinomas and adenomas combined) and those observed with MBC in Swiss SPF mice (i.e., hepatoblastomas and neoplastic nodules) generally occurred at doses which were either lower than or approximately near maximum tolerated dose (MTD) levels. (See discussions of MTD levels for each study in sections D.1., D.2., and D.3.).

5. Oncogenic responses to Benomyl and MBC in Charles River CD-1 mice and Swiss SPF Swiss mice occurred only in the liver; no other type of organ or tissue exhibited an oncogenic response.

6. MBC was not oncogenic in HOE NMRKf (SPF 71) mice. This strain of mouse differs from Charles River CD-1 and Swiss SPF mice in that it normally exhibits a low background incidence rate of liver tumors (Weisse et al., Z. Versuchstierk 17: 91, 1975). In addition, neither Benomyl nor MBC were oncogenic in studies in Charles River CD rats.

7. Benomyl and/or MBC produced positive mutagenic effects that were consistent with adverse effects on the cellular spindle apparatus. These included nondisjunction in A. nidulans, sister chromatid exchange in CHO cells, and micronuclei formation in mouse bone marrow cells. In contrast, equivocal results (both positive and negative findings) for gene mutation were found in Ames tests and mouse lymphoma tests, and negative results for DNA repair were found in primary rat and mouse hepatocyte cultures. The pattern of mutagenicity results appeared to correlate poorly with heritable spindle effects or point mutagenicity.

8. Benomyl was teratogenic following oral (gavage) administration in rats (e.g., microphthalmia) and mice (e.g., cleft palate, supernumary ribs, subnormal vertebral centrum), and also evoked embryotoxic effects in these species. The Committee noted a possible correlation between these effects and the ability of benomyl to act as a spindle poison.

9. Benomyl and MBC are structural congeners of other benzimidazole compounds (e.g., fenbendazole and albendazole) that are currently under review by the FDA Center for Veterinary Medicine; no final determination of oncogenicity has been made by the FDA at this time for these analogues.

G. Classification of Oncogenic Potential:

The Committee concluded that the data available for Benomyl and its primary metabolite, MBC, provides limited evidence of oncogenicity for both chemicals in male and female mice. Criteria contained in the proposed EPA Guidelines (CFR, November 23, 1984) for classifying a carcinogen in either Category B_2 or C were considered. Benomyl and MBC met some of the criteria specified for the B_2 classification. That is, both Benomyl and MBC produced an increased incidence of malignant or combined malignant and benign tumors of the liver. In the case of MBC, tumors were produced in multiple strains of mice (closely related CD-1 and Swiss SPF strains) and in multiple experiments. Furthermore, MBC did produce an unusual type of hepatocellular tumor (hepatoblastoma) but only in male Swiss SPF mice.

Alternatively, the panel considered the guideline criteria for Category C (limited evidence of carcinogenicity), and classified Benomyl in this category for the following reasons: (1) The oncogenic responses observed with Benomyl and MBC were confined solely to the mouse liver, even with repeated experiments; (2) the liver tumors produced by Benomyl and MBC were observed in 2 related strains of mice (CD-1 and Swiss SPF) known to have high background incidence rates of liver tumors whereas no liver tumors were produced by MBC in

another strain of mice [HOE NMRKf (SPF 71)] known to have a low background incidence rate of liver tumors; (3) Benomyl and MBC produced weak mutagenic effects consistent with spindle poison activity rather than gene mutation or DNA repair activity; the committee considered this pattern of mutagenic activity to correlate better with the observed teratogenic effects of Benomyl than with the oncogenic responses to Benomyl and MBC. Because of these tactors the Committee determined that there was insufficient evidence for the B_2 category and therefore, in conformity with the EPA Guidelines noted above, classified both Benomyl and its primary metabolite, MBC, as Category C (possible human) carcinogens.

#9 2/6/86
rew: 3/19/86

3/26/86

MEMORANDUM

SUBJECT: Definition and Use of the Term "MTD" (Maximum Tolerated Dose)

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) RBJ 3/26/86

My signature acknowledges concurrence with the peer review on Benomyl/MBC providing the use of the term "MTD" in this document is consistent with the definition and use as given in: (1) HED SEP: Oncogenicity Potential (Guidance for Analysis and Evaluation of Long Term Rodent Studies) (EPA-540/9-85-019, June 1985); (2) Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation, DHHS (August 17, 1984); and (3) Chemical Carcinogens; A review of the Science and its Associated Principles, February 1985, Office of Science and Technology Policy (FR/Vol. 50, No. 50/March 14, 1985).

MEMORANDUM November 18, 1999

SUBJECT: REVISED Benomyl/MBC Quantitative Risk Assessment (Q_1^*) Based On CD-1 Mouse Dietary Study Using mg/kg b.w.$^{\wedge 3/4}$'s/day Cross Species Scaling Factor

P.C. Code 099101

TO: Deborah Smegal, Toxicologist
Reregistration Branch 3
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: William L. Burnam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

The upper bound estimate of unit risk, $Q_1^*(mg/kg/day)^{-1}$, of Benomyl/MBC based upon female mouse liver adenoma and/or carcinoma combined tumor rates is 2.39×10^{-3} in human equivalents. The dose levels used from the 105-week dietary study were 0, 500, 1500, and 7500 ppm of MBC. The corresponding tumor rates were 1/74, 9/70, 20/75, and 15/75, respectively.

Background

On January 25, 1989, the Cancer Peer Review Committee classified Benomyl/MBC as a Group C - possible human carcinogen, and recommended that, for the purpose of risk characterization, a low dose extrapolation model be applied to the experimental animal tumor data for quantification of human risk (Q_1^*). A Q1* was generated using mg/kg b.w.$^{\wedge 2/3}$'s/day cross species scaling factor (MBC(INE-965) - Qualitative and Quantitative Risk Assessment, CD-1 Mouse Study, E. Fisher, 5/10/89). This revised memo has been generated to reflect the Agency policy change from use of the $^2/_3$'s to the $^3/_4$'s scaling factor

in 1994[1].

All unit risks have been converted from animals to humans by use of the $^3/_4$'s scaling factor (Tox_Risk program, Version 3.5, K. Crump, 1994)[1]. For the conversion to human equivalents, weights of 0.03 kg for the mouse and 70 kg for humans were used.

It is to be noted that the Q_1^* $(mg/kg/day)^{-1}$ is an estimate of the upper bound on risk and that, as stated in the EPA Risk Assessment Guidelines, "the true value of the risk is unknown, and may be as low as zero."

Dose-Response Analysis

The statistical evaluation of mortality (MBC(INE-965) - Qualitative and Quantitative Risk Assessment, CD-1 Mouse Study, B. Fisher, 5/10/89) indicated no significant incremental changes with increasing doses of MBC in female mice. The unit risk, Q_1^*, was obtained by the application of the Multi-Stage model (Tox_Risk program, Version 3.5, K. Crump, 1994).

Female mice had a significant increasing trend at $p < 0.05$, and significant differences in the pair-wise comparisons of all dosed groups (500, 1500 and 7500 ppm) with the controls at $p < 0.01$, for liver adenoma and/or carcinoma tumors combined.

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[1]See memo - Deriving Q_1^*s Using the Unified Interspecies Scaling Factor, P.A. Fenner-Crisp, Director, HED, 7/1/94.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANC

Subject: MBC(INE-965) - Qualitative and Quantitative Risk Assessment, CD-1 Mouse Study (re-evaluation)
caswell no. 79C

From: Bernice Fisher, Biostatistician Bernice Fisher 5/10/89
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

To: Marion P. Copley, D.V.M., Section Head
Review Section II
Toxicology Branch I - Insecticides/Rodenticides
Health Effects Division (H7509C)

Thru: John A. Quest, Ph.D., Section Head John A. Quest 5/15/89
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Summary

The estimated unit risk, Q_1^* of benomyl is $4.20 \times 10^{-3}(mg/kg/day)^{-1}$ in human equivalents. This estimate of Q_1^* is based upon the outcome of the re-evaluation of hepatocellular(adenoma and/or carcinoma) tumors in CD-1 female mice with dose levels of 0, 500, 1500, and 7500 ppm.

This unit risk is essentially at the same level as the previously reported ($Q_1^* = 3.9 \times 10^{-3}(mg/kg/day)^{-1}$ in human equivialents, - Benomyl Risk Assessment for $Q_1^* = 3.9 \times 10^{-3}$ for Carcinogenicity Potency, B.Litt - 3/86). The only difference in the two analysis is the modification of the denominators of tumor rates in female mice, used in the qualitative and quantitative risk assessment. Currently the denominators include only animals at risk (i.e. the total number of animals that were examined with the exclusion of those that died during the first year).

Background

The Peer Review Committee on Benomyl/MBC, January 25,1989 recommended a re-evaluation of the MBC study in CD-1 female mice for the qualitative and quantitative risk assessment. This current evaluation used the collection of individual animal data and then the application of the Statox program to obtain statistical outcomes on survival, tumorgenicity and a unit risk analysis.

The 2 year CD-1 mouse study was conducted by Haskell Labs for E.I. duPont de Nemours and Company, Inc. and reported in January 26, 1982. The mice were assigned in a random manner to the following groups:

Table 1. MBC, CD-1 Mouse, Experimental Design of the Dietary Study

Dose (ppm)	Number of Males	Females	weeks on Study
0	80	80	104
500	80	80	104
1500	80	80	104
7500	80[a]	80	104[a]

a due to the high mortality of males during weeks 52-64 in the high dose group, the dose was reduced to 3750 ppm at week 66 for males and the remaining animals were sacrificed at week 74 instead of 105.

Survival Analysis

In male mice there was a significant ($p<.001$) increasing trend in mortality with dose increments of MBC. There also was a significant ($p<.05$) diference between controls and the high (7500-3750 ppm) dose group as well as a significant ($p<.01$) difference between the mid (1500 ppm) dose group and controls (Table 2).

In the females, there was no statistical evidence of dose related mortality either in the trend analysis or in the the pair-wise comparison of control and each dose group (Table 3).

The statistical evaluation of mortality in the mouse was based upon the Thomas, Breslow and Gart computer program.

Table 2. MRC - Male Mouse Study, Mortality Rates+ and Cox or Generalized K/W Test Results

Dose(ppm)	Week 1-26	27-52	53-73[a]	74-104[b]	Total
0	1/80	3/79	25/76	33/51	62/80 (78)**
500	0/80	8/80	33/72	24/39	66/80 (83)
1500	0/80	9/80	36/71	26/35	71/80 (89)**
7500-3750[c]	4/80	12/76	41/64	---	57/80 (71)*

+ Number of animals that died during interval/ Number of animals alive at the beginning of the interval.

( ) percent

a Final Sacrifice at week 74 for highest (7500-3750 ppm) dose group.

b Final Sacrifice at week 105 for 0, 500, and 1500 ppm dose groups.

c Dose reduced from 7500 to 3750 ppm at week 66 in highest dose group.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ and if ** then $p<.01$.

Table 3. MBC - Female Mouse Study, Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose(ppm)	Week 1-26	27-52	53-78	79-104[a]	Total
0	3/81	4/78	26/74	26/48	59/81 (73)
500	4/79	6/75	17/69	36/52	63/79 (80)
1500	2/80	3/78	27/75	34/43	66/80 (83)
7500	2/80	2/78	23/76	32/53	59/80 (74)

[+] Number of animals that died during interval/ Number of animals alive at the beginning of the interval.

( ) percent

a Final Sacrifice at week 105

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ and if ** then $p<.01$.

Tumor Analysis

In mice, both sexes had elevated tumors in the liver with dose increments of benomyl.

In the males, with dose related significant mortality, the Peto Prevalence method was used to evaluate tumor trends and the pair-wise comparison with controls and each dose group. In addition, tumorgenicity in the highest (7500-3750ppm) dose group was not analysed because of high mortality and thus the lack of sufficient animals for justifiable statistical evaluation. The results indicated that there was a significant ($p=.01$) increasing trend in hepatocellular carcinoma tumor rates and a significant ($p=.005$) increasing trend in the combined hepatocellular (adenoma and/or carcinoma) tumor rates with incremental doses of benomyl. In the pair-wise comparison of of controls and the mid (1500 ppm) dose group, there was a significant ($p=.012$) difference in liver carcinoma tumor rates and also a significant ($p=.007$) difference in the combined liver (adenoma and/or carcinoma) tumors. In the pair-wise comparison of control and the low (500 ppm) dose group, there was a significant ($p=.009$) difference in the combined liver (adenoma and/or carcinoma) tumors (Table 4).

In this qualitative risk analysis of female mice, the denominators for liver tumor rates included only animals at risk. By definition it included all animals examined, less those that died during the first year of the study. While in the previous risk assessment - Statistical Evaluation and Oncogenicity Risk Assessment of Benomyl, Benlate, and MBC 2-Year Feeding Studies in Mice, R.Litt, 5/82 - all animals that were examined were included in the denominator without exception. In female mice, not having significant dose related mortality, the Cochran-Armitage trend test and the Fisher Exact test for pair-wise comparisons was used to evaluate liver tumor data. The outcome of these tests indicated a significant ($p=.010$) dose related trend in liver carcinoma tumor rates and also a significant ($p=.019$) dose related trend in the combined liver (adenoma and/or carcinoma) tumors. In the pair-wise comparison of controls and the highest (7500 ppm) dose group there was a significant ($p<.001$) difference in combined liver (adenoma and/or carcinoma) tumors and also a significant ($p=.001$) difference in liver carcinomas. In the pair-wise comparison of control and the mid (1500 ppm) dose group there was a significant difference in liver adenomas ($p=.030$) and in liver carcinomas ($p<.001$) and in the combined liver (adenoma and/or carcinoma) tumors ($p<.001$). In addition the pair-wise comparison of controls and the lowest (500 ppm) dose group resulted in a significant difference in the combined liver (adenoma and/or carcinoma) tumors ($p=.007$) and in liver adenoma tumors ($p=.025$) (Table 5).

Table 4. MBC - Male Mice, Hepatocellular Tumor Rates+ and the Peto Prevalence Test Results

	Dose(ppm)			
Liver Tumor	0	500	1500	7500-3750[a]
Adenoma	11/76 (14)	15/72 (21)	14/73 (19)	3[b]/67 (4)
p=	0.155	0.072	0.131	—[c]
Carcinoma	2/76 (3)	5/72 (7)	9[d]/73 (12)	0/67 (0)
p=	0.010*	0.080	0.012**	—[c]
Combined Tumors	13/76 (17)	20/72 (28)	23/73 (32)	3/67 (4)
p=	0.005**	0.009**	0.007**	—[c]

+ Number of tumor bearing animals/ Number of animals at risk (excluding those that died before 52 weeks).

( ) percent

a 7500 ppm dose reduced to 3750 ppm at week 66.
b first adenoma observed at week 62.
c animals at high dose (7500-3750 ppm) were not evaluated because of early high mortality and subsequent final sacrifice at week 74.
d first carcinoma observed at week 88.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ and if ** then $p<.01$.

Table 5. MRC - Female Mice Hepatocellular Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dose(ppm)			
Liver Tumor	0	500	1500	7500
Adenoma	0/74 (0)	5/70 (7)	5/75 (7)	3[a]/75 (4)
p=	0.441	0.025*	0.030*	0.125
Carcinoma	1/74 (1)	4/70 (6)	15[b]/75 (20)	12/75 (16)
p=	0.010*	0.166	0.000**	0.001**
Combined Tumors	1/74 (1)	9/70 (13)	20/75 (27)	15/75 (20)
p=	0.019*	0.007**	0.000**	0.000**

[+] Number of tumor bearing animals/ Number of animals at risk (excluding those that died before 52 weeks).

( ) percent

a first adenoma observed at week 90.
b first carcinoma observed at week 77.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ and if ** then $p<.01$.

Dose-Responce Analysis

The most sensitive measurable reaction to benomyl occurred in female mice in terms of significant dose related trends and pair-wise significant differences between controls and selected dose levels in liver tumors. Since there was no statistical evidence of significant dose related mortality in the females, the estimate of unit risk, Q_1^* of benomyl, based upon the liver tumor data, was calculated by the use of Global86 (Multi-Stage process) computer program of K.Crump.

The unit risk calculated from the female mouse liver tumor data in ppm doses was converted to mouse mg/kg/day by the use of Lehman's Tables and then to human equivalents by the use of interspecies surface area adjustments as recommended by EPA Cancer Guidelines (1986).

The resultant estimate of Q_1^* is as follows:

Female liver tumors (adenomas &/or carcinomas)	Mouse, Q_1^* $(mg/kg/day)^{-1}$	In Human Equivalents
	3.14×10^{-4}	4.20×10^{-3}

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION. PESTICIDES AND
TOXIC SUBSTANCES

March 30, 2000

Memorandum

SUBJECT: Toxicology Chapter for Benomyl and Carbendazim. DP Barcode D264602, Case 819338, Benomyl PC Code 099101, Carbendazim PC Code 128872.

FROM: Deborah Smegal, M.P.H. Toxicologist
Re-Registration Branch 3
Health Effects Division (7509C)

Deborah Smegal

THRU: Jess Rowland, M.S., Branch Chief
Re-registration Branch 3
Health Effects Division (7509C)

Deborah Smegal for J. R

TO: Demson Fuller, Chemical Review Manager
Special Review and Reregistration Division (7508C)

This memorandum summarizes the guideline studies submitted by the registrant, and other relevant toxicity studies considered by HED in developing the acute and chronic reference doses (RfDs) and toxicity endpoints for use in risk assessment for benomyl and its primary metabolite, carbendazim (Methyl 2-Benzimidazole Carbamate or MBC).

Table 2. Subchronic Toxicity of Benomyl			
GDLN	STUDY	DOSE (mg/kg/day) (1)	RESULTS (mg/kg/day) (1)
870.3200 (82-2)	21-Day Dermal Toxicity Study in Rabbits MRID #: 00097287 (Hood et al. 1969) Core Grade: acceptable guideline	0, 50, 250, 500, 1000 and 5000 (Doses already adjusted for % a.i. in study)	**53% a.i benomyl** NOAEL: 500 LOAEL: 1000 Effects: Males of the 1000 mg/kg/day group exhibited 30% and 24% decreases in testicular weight and testes-to-body weight ratios, respectively (both not statistically significant), which was not apparent at 5000 mg/kg/day. However, lack of testicular effects at 5000 mg/kg/day may be due to a low number of animals (2/sex) evaluated at this dose. Females exposed to 1000 and 5000 mg/kg/day exhibited diarrhea, oliguria and hematuria. Moderate skin irritation was reported for all dose groups.
870.3465 (82-4)	Subchronic Inhalation in Sprague-Dawley Rats (90 days) MRID #: 40399501 (Warheit 1987) Core Grade: acceptable guideline	Males: 0.96, 4.8 or 19.2 mg/kg/day Females: 1.4, 7.0 or 28.8 mg/kg/day (0, 10, 50 or 200 mg/m^3, or 0, 0.01, 0.05 or 0.2 mg/L) 4 hr/day	**95% a.i. benomyl** NOAEL: 0.96 (males) LOAEL: 4.8 (males) Effects: At 4.8 mg/kg/day olfactory degeneration was characterized by necrosis, chronic and acute inflammation and loss of olfactory epithelium with foci of repair. Males exposed to 19.2 mg/kg/day had decreased body weights (10.8%) and body weight gains (13.6%).

(1) Unless specified, mg ai benomyl/kg/day.
NOAEL = No Observable Adverse Effect Level
LOAEL = Lowest Observable Adverse Effect Level
SGPT = Serum Glutamic Pyruvic Transaminase

c. Chronic Toxicity and Carcinogenicity

Benomyl was evaluated for carcinogenic potential in both rats, and mice. In addition, benomyl was evaluated for chronic toxicity in dogs. In dogs and mice, the most sensitive toxicological endpoint is liver toxicity that occurred at levels as low as 62.5 mg/kg/day. Dogs appear to be the most sensitive species for liver toxicity following chronic oral exposure. Liver effects were characterized by hepatic cirrhosis, bile duct proliferation with corresponding biochemical changes indicative of liver injury. Benomyl induced liver tumors (hepatocellular carcinomas) in mice. There is no evidence of carcinogenicity in rats. Testicular effects in mice were characterized as degenerative changes in the testes and epididymides at very high doses of 1125/750 mg/kg/day. Benomyl is classified in group C (possible human carcinogen). HED

calculated a Q1* of 2.39×10^{-3} $(mg/kg/day)^{-1}$ for both benomyl and MBC based on a mouse carcinogenicity study with MBC.

The following tables summarize the chronic toxicity/carcinogenicity studies for benomyl:

Table 3. Chronic Toxicity/Carcinogenicity of Benomyl

GDLN	STUDY	DOSE (mg/kg/day)	RESULTS (mg/kg/day)
870.4100a 870.4200 (83-1(a) 83-2)	Chronic feeding study in CD rats Accession # 051427 Sherman et al. 1969 Core Grade: minimum	0, 5, 25, or 125 (0, 100, 500 or 2500 ppm) (Doses adjusted for % a.i.)	**51 or 72.2% a.i. benomyl** NOAEL: >125 (HDT) LOAEL: none established Effects: None observed. No evidence of carcinogenicity. Deficiencies: Limited clinical chemistry analysis, and only 36 rats/sex/dose were evaluated.
870.4100b (83-1b)	Chronic feeding study in beagle dogs (2 yrs) MRID # 00061618, 00081913, 0097305, 00097318, 00097326 Sherman et al. 1970 Core Grade: acceptable guideline	0, 2.5, 12.5, or 62.5 (0, 100, 500 and 2500 ppm) (Doses adjusted for % a.i.)	**50% a.i. benomyl** NOAEL: 12.5 LOAEL: 62.5 (HDT) Effects: At 62.5 mg/kg/day effects include hepatic cirrhosis, bile duct proliferation, testicular degeneration, as well as decreased weight gain and food consumption. Males had increased in cholesterol, alkaline phosphatase and SGPT and decreased total protein and albumin/globulin (A/G) ratio, which were correlated with chemically-induced hepatic injury. Focal testicular degeneration was present in all treatment groups, with marked testicular degeneration (reduced testes weight, absence of spermatozoa and spermatic giant cells) in 1/3 dogs at 62.5 mg/kg/day.

Table 3. Chronic Toxicity/Carcinogenicity of Benomyl			
GDLN	STUDY	DOSE (mg/kg/day)	RESULTS (mg/kg/day)
870.4200b (83-2b)	Chronic feeding study in CD-1 mice (2 yrs) MRID # 00096514 Schneider et al. 1982 Core Grade: acceptable guideline	0, 75, 225 or 1125 (750) (0, 500, 1500 and 7500 ppm) (the 7500 ppm dose level was reduced to 5000 ppm after week 37).	99, 99.2% a.i. benomyl NOAEL: none LOAEL: 75 Effects: significant increase in hepatocellular carcinomas in both males and females at 75 mg/kg/day. There was also an increase in the combined incidence of hepatocellular adenomas and carcinomas in mid and high dose females. At the highest dose tested, the testes and epididymides showed degenerative changes, which were characterized by degeneration of the seminiferous tubules, atrophy and tubular degeneration.

HDT = Highest Dose Tested
SGPT = Serum Glutamic Pyruvic Transaminase

Chronic/Carcinogenicity Study in Rats

In a study conducted in 1969, benomyl (51.5 or 72.2%) was administered in the diets of CD rats (36/sex/dose) at levels of 0, 100, 500 or 2500 ppm for two years (Accession # 051427, Sherman et al. 1969). This is approximately equivalent to 0, 5, 25 or 125 mg ai benomyl/kg/day. Six rats/sex/dose were sacrificed and examined for gross and microscopic pathology at 1 year. After two years, the surviving rats were also sacrificed. All tissues from the brain, heart, kidney, adrenal, stomach, liver, spleen, testes and lung were examined microscopically in the control and high dose group at both sacrifices, while all tissues were examined only at the 2 year sacrifice in the low and mid dose groups. In addition, histopathology was conducted on a more comprehensive list of organs in the control and high-dose groups at 1 and 2 years, but only for the liver, kidney and testes of the low and mid-dose groups at 2 years.

There were no treatment-related effects on mortality, body weight, food consumption, organ weights, clinical chemistry, urinalysis, gross pathology or histopathology. Only two clinical chemistry parameters, alkaline phosphatase (AP) and serum glutamic-pyruvic transaminase (SGPT), were evaluated, which makes it difficult to confirm that there were no adverse effects. There were no treatment-related organ weights or histopathologic changes in any of the groups tested at either the 1 (6 rats/sex/dose) or 2 year (30 rats/sex/dose) sacrifices. Therefore, the toxicological significance of any clinical chemistry alterations is questionable in the absence of corroborative changes in organ weight and histopathology in the liver. Liver changes and testicular degeneration were a frequent occurrence, but were equally spread between control and test groups. There was no evidence of carcinogenicity.

This study was conducted in 1969, prior to the 1984 Subdivision F guidelines for a chronic toxicity study (83-1) and chronic feeding/oncogenicity study (83-2), and therefore is classified as minimum (i.e., does not meet current evaluation standards, however, is adequate for risk assessment). Deficiencies include limited clinical chemistry analysis, failure to identify a LOAEL, the maximum tolerated dose (MTD) was not established and only 36 rats/sex/dose were evaluated (when 50/sex/dose are currently required for a carcinogenicity study).

Chronic/Carcinogenicity Study in Mice

Benomyl (99%, 99.2%) was administered in the diets of CD-1 mice (80/sex/dose) at levels of 0, 500, 1500 and 7500/5000 ppm (the 7500 ppm dose level was reduced to 5000 ppm after week 37) for two years. This is equivalent to 0, 75, 225 or 1125/750 mg/kg/day. Hepatocellular carcinomas were significantly elevated in male (500 and 1500 ppm) and female (500 and 7500/5000 ppm) mice. In addition, the combined incidence of hepatocellular adenomas and carcinomas were significantly elevated in males (500 and 1500 ppm) and females (500 and 7500/5000 ppm). The tumorigenic response appeared to be compound-related (e.g., they occurred with significant positive trends, and the incidence exceeded historical rates). Pulmonary alveologenic carcinomas were significantly elevated in the low and mid dose males, but not at the high dose. Therefore, the pulmonary tumors did not appear to be compound-related since there was no dose-response and the observed incidences were within the range for historical control rates at the laboratory. At the highest dose tested, the testes and epididymides showed degenerative changes (aspermia), which were characterized by degeneration of the seminiferous tubules, atrophy and tubular degeneration.

There was a statistically significant decrease in body weight reported for both males and females in the highest dose tested. The body weights were approximately 10% lower than controls in both sexes from weeks 13 to study termination at week 104. Sporadic decreases in body weights were also reported at one or two weighing intervals in mice receiving 1500 ppm but did not appear to be statistically or biologically significant. The highest dose of benomyl tested in male mice appeared to exceed the maximum tolerated dose (MTD). High dose males exhibited an approximate 10% decreased body weight gain, hepatocellular toxicity (e.g., foci of cellular alteration, cytomegaly, and foci of degeneration) and degenerative changes in the testes. The high dose did not produce tumors in males, possibly because of the hepatocellular toxic changes that were observed (e.g., the observed liver toxicity may have altered the ability of benomyl the be metabolized to MBC).

This study is **acceptable** and satisfies the requirement for a carcinogenicity study in mice. Based on the reported decreases in body weight and the increase in incidence of liver tumors, the dose selection in this study appears to be adequate (MRID 00096514).

Chronic Toxicity Study in Dogs

Groups of 4/sex/dose beagle dogs were administered a formulated product containing benomyl in

the diet at dosage levels of 0, 100, 500 and 2500 ppm for 2 years (MRID 00061618). The dietary concentrations are equivalent to 0, 2.5, 12.5 and 62.5 mg/kg/day ai benomyl. After one year, one dog/sex from control and high dose groups were sacrificed. Organ weights, gross necropsy and histopathological evaluations were conducted after two years. Only the livers and testes were examined histologically in the 100 and 500 ppm groups.

There were no treatment-related effects on mortality, hematology, urinalysis, or clinical signs. Body weight gain and food consumption were decreased in the high dose group. Males in the high dose group had increased cholesterol, alkaline phosphatase and glutamic-pyruvic transaminase (GPT) values, as well as decreased total protein and albumin/globulin (A/G) ratio. Similar effects, other than cholesterol and total protein, were noted in the high dose females. The clinical chemistry observations support the adverse liver effects in the high dose group, characterized as cirrhosis (one male at 1 year and 2 males and 1 female at 2 year sacrifice) and slight to marked bile duct proliferation in 4/6 dogs of the 2500 ppm (62.5 mg/kg/day ai) group. Focal testicular degeneration was present in all treatment groups, with marked testicular degeneration (reduced testes weight, absence of spermatozoa and spermatic giant cells) in 1/3 dogs at 2500 ppm.

The NOAEL is 500 ppm (12.5 mg/kg/day ai) based on hepatic cirrhosis, clinical chemistry alterations, testicular degeneration as well as decreased weight gain and food consumption noted at 2500 ppm. This study is **acceptable** and satisfies the guideline requirements for a chronic dog study.

Classification of Carcinogenic Potential

Both benomyl and MBC are classified as group C chemicals (possible human carcinogens) by the Cancer Peer Review Committee. On 5/21/86, the Scientific Advisory Panel (SAP) concurred with the classification of benomyl. The rationale for this classification is as follows: (1) the carcinogenic response for both benomyl and MBC are confined solely to the mouse liver, even with repeated experiments; (2) the liver tumors produced by benomyl and MBC were observed in 2 related strains of mice (CD-1 and Swiss SPF) know to have high background incidence rates of liver tumors, whereas no liver tumors were produced by MBC in another strain of mice [NMRKf (SPF 71)] known to have a low background incidence rate of liver tumors (see discussion under Section III Carbendazim Carcinogenicity Discussion); (3) benomyl and MBC produced weak mutagenic effects consistent with spindle poison activity rather than gene mutation or DNA repair activity.

The Peer Review Committee noted the occurrence of mostly malignant hepatocellular tumor response with MBC in two stains of mice, and the presence of unusually occurring and malignant hepatoblastomas with MBC in male SPF Swiss mice. In addition, the mutagenicity information indicates that the aneuploidy known to be produced by benomyl could theoretically result in a loss of tumor suppressor genes and a potential oncogenic effect.

HED estimated a unit risk Q_1^* of 2.39×10^{-3} $(mg/kg/day)^{-1}$ for both benomyl and MBC. This estimate is based on the outcome of the re-evaluation of the hepatocellular (adenoma and/or carcinoma) tumors in CD-1 female mice with dose levels of 0, 500, 1500 or 7500 ppm MBC (Wood et al. 1982). The Q_1^* was calculated using the $(mg/kg/day)^{3/4}$ species scaling factor. Details of the quantitative estimate are presented in Attachment 1 of this memorandum.

d. Developmental Toxicity

Benomyl was evaluated for developmental toxicity in rats and rabbits in registrant-submitted studies. In rats, developmental effects were noted at doses ranging from 62.5 to 125 mg/kg/day in the absence of maternal toxicity. At 62.5 mg/kg/day effects included increased incidence of ocular malformations (microphthalmia and anophthalmia), increased fetal mortality and reduced fetal weight. Effects at 125 mg/kg/day included increased incidence of malformations of the brain, characterized by distended lateral ventricles and hydrocephaly. Fetuses of rabbit does exposed to 180 mg/kg/day developed a significant increase incidence in visceral variations (small renal papillae) that were not readily attributed to exposure and were not considered to be malformations because they may have occurred as a result of incomplete maturation. Nevertheless, the visceral variations occurred at maternally toxic doses as indicated by stained tails and reduced feed consumption at 180 mg/kg/day.

Literature studies have also demonstrated that benomyl induces developmental effects in rats and mice following gavage administration to pregnant animals at doses as low as 62.5 mg/kg/day and 100 mg/kg/day, respectively (Kavlock et al. 1982, Chernoff 1985). Developmental effects in rats include microphthalmia, decreased fetal weight, increased fetal mortality and delayed skeletal and visceral maturation, while effects in mice include cleft palate, supernumerary ribs and subnormal vertebral centrum. Literature studies have also demonstrated a differential in fetal response to gavage versus dietary exposure to benomyl, with gavage dosing producing anomalies at approximately one-tenth of the dietary dose (Kavlock et al. 1982, Chernoff 1985). In addition, benomyl caused sustained adverse effects on the male reproductive system in a postnatal rat study at doses as low as 31.2 mg/kg/day (Kavlock et al. 1982).

The following table summarizes the developmental studies for benomyl:

c. Chronic Toxicity and Carcinogenicity

Carbendazim was evaluated for carcinogenic potential in both rats, and mice. In addition, carbendazim was evaluated for chronic toxicity in dogs. In all animal species, the most sensitive toxicological endpoint is liver toxicity that occurred at levels as low as 12.5 mg/kg/day. Dogs appear to be the most sensitive species for liver toxicity following chronic oral exposure. Carbendazim induced liver tumors (hepatocellular carcinomas) in mice. There is no evidence of carcinogenicity in rats, however, the rat study only tested 36 rats/sex/dose (and only 20/sex/dose in the 250 mg/kg/day dose group) (when current guidelines require 50 rats/sex/dose). The following table summarizes the chronic toxicity/carcinogenicity studies for carbendazim:

Table 11.
Chronic Toxicity/Carcinogenicity of Carbendazim

GDLN	STUDY	DOSE (mg/kg/day)	RESULTS (mg/kg/day)
870.4100 870.4200 (83-1& 2)	Chronic feeding/ carcinogenicity study in CD rats (2 yrs) MRID # 00088333 Accession #: 232870-0, 232871 Sherman et al. 1972 Core Grade: minimum	0, 5, 25, 250 or 125/500 (430) [0, 100, 500, 5000 or 2500/10000 (8557) ppm]	**53% a.i. carbendazim** NOAEL:25 LOAEL: 250 Effects: Statistically significant decreases in red blood cell parameters (hematocrit, hemoglobin an red blood cells) in females and histological lesions in the liver (cholangiohepatitis and pericholangitis) in males and females. No evidence of carcinogenicity. Note: Dietary levels in 2,500 ppm were increased to 7,500 ppm at 18 weeks and to 10,000 ppm from weeks 20-104 for a time-weighted average of approximately 8557 ppm (430 mg/kg/day). Deficiencies: Only 36 rats/sex/dose tested (only 20 rats/sex were in 250 mg/kg/day dose group). Lack of complete clinical chemistry data and histopathology examination. At 24 months, only liver evaluated in 5 and 25 mg/kg/day groups and only liver, kidney and testes evaluated in 250 mg/kg/day group.

Table 11. Chronic Toxicity/Carcinogenicity of Carbendazim			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.4100b (83-1b)	Chronic feeding study in beagle dogs (2 yrs) MRID # 00088333 Accession #: 232870-0, 232871 (Sherman et al. 1972) Core Grade: acceptable guideline	0, 2.5, 12.5, or 37.5/62.5 (0, 100, 500 and 1500/2500 ppm) (Doses adjusted for % a.i.)	**53% a.i. carbendazim** NOAEL: 2.5 LOAEL: 12.5 Effects: At 12.5 mg/kg/day swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis and biochemical alterations indicative of liver damage (i.e., increased cholesterol, total protein, SGPT and alkaline phosphatase levels, and decreased A/G ratio). At 37.5/62.5 mg/kg/day, anorexia, distended abdomens and poor nutritional condition were reported.
870.4100b (83-1b)	Chronic feeding study in beagle dogs (1 yr) Accession # 265664 (Stadler et al. 1986) Core Grade: acceptable guideline	F:0, 2.93, 6.43 or 16.54 mg/kg M: 0, 3.2, 7.19, 17.07 (0, 100, 200, or 500 ppm)	**98.8% a.i. carbendazim** NOAEL: 6.43 (200 ppm) LOAEL: 16.54 (500 ppm) Effects: Possible transient increase in cholesterol (males and females) consistent with previous dog feeding studies.
870.4200b (83-2b)	Chronic feeding study in CD-1 mice (2 yrs) MRID # 256028, and 256029 Wood et al. 1982 Core Grade: acceptable guideline. The study was designed to specifically evaluate the liver carcinogenicity potential of MBC	0, 75, 225, 1125 (females) or 1125/563 (males) (0, 500, 1500 or 7500 (females) or 7500/3750 (males) ppm)	**99.3% a.i. carbendazim** NOAEL (non-cancer systemic): 75 LOAEL (non-cancer systemic): 225 Effects: liver toxicity (hepatocellular necrosis and swelling), body weight decrease and lymphoid depletion. In both sexes, there was an increased incidence of liver tumors. In males, hepatocellular carcinomas were noted at 225 mg/kg/day, while females exhibited carcinomas and adenomas at all dose levels. Note: The 7500 ppm was reduced to 3750 ppm at 66 weeks in males due to increased mortality.

Table 11.
Chronic Toxicity/Carcinogenicity of Carbendazim

GDLN	STUDY	DOSE (mg/kg/day)	RESULTS (mg/kg/day)
870.4200b (83-2b)	Chronic feeding/ carcinogenicity study in NMRKf mice (2 years) MRID # 2560302 (Donaubauer et al. 1982) Core Grade: unacceptable guideline	0; 5.8-7.1; 17.1 - 21.2; 34.4 - 41.9 or 522 - 648 (0, 50, 150, 300 or 1000/5000 ppm)	**99% a.i. carbendazim** NOAEL (non-cancer systemic): 34.4 - 41.9 LOAEL (non-cancer systemic): 522 - 648 Effects: increases the incidences of hepatic cell hypertrophy, clear cell foci and hepatocellular necrosis. No increased incidence of carcinogenicity was noted. Note: The 1000 ppm dose was increased to 2000 ppm after 4 weeks and to 5000 ppm after an additional 4 weeks. Deficiencies: incomplete examination of most recommended tissues, blood and urine were not collected for analysis.
870.4200b (83-2)	Chronic feeding/ carcinogenicity study in Swiss mice (80 weeks) MRID # 256029 (Beems et al. 1976) Core Grade: unacceptable guideline	0, 22.5, 45 or 750 (0, 150, 300 or 5000 ppm)	**99% a.i. carbendazim** NOAEL:45 LOAEL:750 Effects: hepatic alterations which included increased relative liver weights in both sexes, increased number of foci of cellular alterations in the liver in females, neoplastic nodules in females and hepatoblastomas in males Deficiencies: Brief methods, there were no historical data or microscopic or gross pathology reports for individual animals, and there was no assurance that the diets were analyzed for compound homogeneity and stability. In addition, there were no hematology or clinical chemistry analysis, nor urinalysis. Only organs or lesions suspected of being tumors and livers (2 sections) were examined histologically.

Chronic/Carcinogenicity Study in Rats

MBC (methyl ester, 53%) was administered in the diets of CRL: CD1 rats (36/sex/dose, except for 20/sex at 250 mg/kg/day group) at dietary levels of 0, 100, 500, 5,000 or 2,500/10,000 ppm (8557 ppm) (equivalent to 0, 5, 25, 250 or 125/500 (430) mg/kg/day)(MRID 00088333, Sherman et al. 1972). The dietary levels were increased in the 2,500 ppm group to a level of 7,500 ppm at 18 weeks and to 10,000 ppm from weeks 20 to study termination at week 104, yielding an approximate time-weighted average (TWA) daily dose of 8557 ppm (430 mg/kg/day) for the 104 week study duration. Treatment was initiated for the 5,000 ppm group 3 weeks late (age 33

weeks of age) without preliminary hematology. There were no apparent treatment related signs of toxicity nor were there any effects on mortality, food consumption or feed efficiency. In females in the highest dose group, there was a decrease in body weight gain when compared to controls at both 15 (14% lower) and 24 months (24% lower).

At 5000 and 2500/10,000 ppm (250 and TWA 430 mg/kg/day), statistically significant decreases in red blood cell counts, hemoglobin and hematocrit values were reported in females at 24 months. Transient, non-significant elevations in SGPT were reported in males and females in the high dose group (TWA 430 mg/kg/day) at 12 months, but these findings were not apparent at 24 months and therefore, were not considered to be related to the administration of benomyl. In males and females receiving 5000 ppm and 2500/10,000 ppm (TWA 8557 ppm) benomyl, there was an increase in the incidence and severity of cholangiohepatitis and pericholangitis. There was no evidence of carcinogenicity reported in this study, however, this study is classified as minimum, and does not meet the Subdivision F chronic toxicity or oncogenicity guidelines (see below). Thus, the NOAEL in this study was 500 ppm (25 mg/kg/day), based on statistically significant decreases in red blood cell parameters and histological lesions in the liver (cholangiohepatitis and pericholangitis) at both 5000 and 2500/10,000 ppm (8557 ppm) (250 and TWA 430 mg/kg/day.

This study was conducted in 1972, prior to the 1984 Subdivision F guidelines for a chronic toxicity study (83-1) and chronic feeding/oncogenicity study (83-2), and therefore is classified as minimum (i.e., does not meet current evaluation standards, however, is adequate for risk assessment). Deficiencies include small sample size (36/sex/dose except 20/sex in 5000 ppm group, when 50/sex/dose are required for 83-2 and current oncogenicity guidelines, limited histopathology (the target organ testes was not evaluated in the two lowest dose groups) and limited clinical chemistry evaluation [i.e., only plasma alkaline phosphatase (AP) and Serum Glutamic Pyruvic Transaminase (SGPT)] only in the two highest dose groups. Based on the observed decreases in body weight at the highest doses tested and the observations of liver lesions and decreases in hematology measurements, the study appears to have been conducted at adequate dose levels. The adequacy of the doses tested is further supported by the results of the 90 day neurotoxicity study, where terminal body weights and body weight gains were decreased when compared to controls at the highest dose tested of 7500 ppm (MRID 00088333).

Chronic/Carcinogenicity Study in Mice

In a carcinogenicity study conducted with MBC (99.3%), the test material was administered in the diets of CD-1 mice (80/sex/dose) at levels of 0, 500, 1500, 7500 (females) or 7500/3750 (males) ppm (equivalent to 0, 75, 225, 1125 (females) or 1125/563 (males) mg/kg/day) (MRID = 256028, and 256029, Wood et al. 1982). In males receiving 7500 ppm, the dose was reduced to 3750 at week 66 and fed until this group was terminated at week 73. All other groups were fed at the designated dietary levels up to week 104. Survival of the male mice in the intermediate (1,500 ppm) and high (7,500-3750 ppm) dose groups was significantly lower than that of male control mice. A 12% decrease in body weight was reported in males receiving 1500 ppm when

compared to controls at week 104. Hepatoxicity, characterized by hepatocellular necrosis and swelling was also reported in males at 1500 ppm. In both sexes, an increased incidence of liver tumors was reported. Heptatocelluar carcinomas, and adenomas and carcinomas combined, were significantly elevated in male mice (mid dose level); no increase in adenomas (alone) occurred in males. The lack of carcinogenic response in high dose males is likely to be explained by either their early deaths or sacrifice at 73 weeks. In female mice there were significant increases in adenomas (low and mid doses), carcinomas (mid and high doses), and adenomas and carcinomas (all 3 dose levels tested) of the liver. No increased incidence of liver hyperplasia occurred in treated mice. Only the carcinomas (mid and high dose levels) and the adenomas/carcinomas combined (all 3 dose levels) in female mice exceeded the historical control response rates. There was a treatment-related decrease in female thymic weight (absolute and relative) and a dose-related decrease in male thymic weight. This was consistent with the treatment-related lymphoid depletion observed in both sexes of the mid and high dose groups.

The high dose level of MBC clearly exceeded the MTD level in male mice (but not females) because of excessive mortality. The mid dose level appeared to approximate the MTD level for males. The non-cancer systemic NOAEL was 500 ppm based on liver toxicity, body weight decrease and lymphoid depletion reported at 1500 ppm (MRID 256028, 256029). This study is **acceptable** and satisfies the 83-2 guidelines for an oncogenicity study in mice.

In another carcinogenicity study in NMRKf mice (100/sex/dose), MBC (99%) was administered at dietary levels of 0, 50, 150, 300 or 1000/5000 ppm for 2 years. The 1000 ppm dose was increased to 2000 ppm after 4 weeks and from 2000 ppm to 5000 ppm after an additional 4 weeks on the study. Dietary concentrations were reported to be equal to 0, 5.8, 17.1, 34.4 or 522 mg/kg for males and 0, 7.1, 21.2, 41.9 or 648 mg/kg for females, respectively. This study was designed to specifically address the finding of liver carcinogenicity and all tissues were not subjected to a gross or microscopic examination. The systemic NOAEL was 300 ppm (34.4 - 41.9 mg/kg) and the systemic LOAEL was 5000 ppm (520 - 650 mg/kg) based on liver toxicity in both sexes which consisted of increases the incidences of hepatic cell hypertrophy, clear cell foci and hepatocellular necrosis. The incidence of carcinogenicity was not increased in this study. The NMRKf strain of mouse, in contrast with the CD-1 and Swiss SPF mice, normally exhibits a low background incidence of liver tumors. Because of the reported inconsistencies in the analysis of MBC and because an incomplete gross and microscopic assessment, this study was classified as **not acceptable** guideline. In addition, blood and urine were not collected for evaluation (MRID 2560302).

Carbendazim was also associated with an increase in the incidence of hepatoblastomas in Swiss mice. MBC (99%) was administered in the diets of SPF Swiss mice (100/sex/dose) at dietary levels of 0, 150, 300 or 5000 ppm (equivalent to 22.5, 45 or 750 mg/kg/day) for 80 weeks (Accession No. 256029, Beems et al. 1976). The systemic NOAEL was 300 ppm (45 mg/kg) and the systemic LOAEL was 5000 ppm (750 mg/kg) based on hepatic alterations which included increased relative liver weights in both sexes, increased number of foci of cellular alterations in the liver in females, neoplastic nodules in females and hepatoblastomas in males.

This study was actually a report and was classified as **unacceptable** guideline because the methods were brief, there were no historical data provided, there were no microscopic or gross pathology reports for individual animals, and there was no assurance that the diets were analyzed for compound homogeneity and stability. In addition, there were no hematology or clinical chemistry analysis, nor urinalysis. Only organs or lesions suspected of being tumors and livers (2 sections) were examined histologically (MRID 256029).

Chronic Toxicity Study in Dogs

Beagle dogs (4/sex/dose) were administered a product formulation containing 53% a.i. carbendazim, a primary metabolite of benomyl at dietary doses levels of 0, 100, 500 or 1500/2500 ppm for two years (MRID 00088333, Accession #: 232870-0,232871, Sherman et al. 1972). Due to weight loss and decreased appetite, the dose to some dogs in the 2500 ppm group was reduced to 1500 ppm. This is equivalent to 0, 2.5, 12.5 or 37.5/62.5 mg/kg/day ai MBC. One dog/sex from control and 500 ppm group, as well as one female from the high dose group was sacrificed at 1 year. One male from the high dose group was sacrificed in extremis after 42 weeks on the test diet. Only the livers and testes were examined histologically in the 100 an 500 ppm dose groups.

There was no mortality reported for the control or 100 and 500 ppm groups. However, three of the males in the high dose group were sacrificed after 22 and 24 weeks because of poor nutrition. No females in the high dose group died. Body weight and food consumption were adversely affected in all high dose group animals. No treatment-related effects were noted in dogs fed 100 ppm (2.5 ai mg/kg/day). Diffuse and marked testes atrophy and aspermatogenesis were observed in 2/4 males of the 100 ppm group, which were not considered treatment-related because these observations were not present in the other dose groups. Dogs of both sexes in the mid and high dose groups (12.5 ai or 37.5/62.5 mg/kg/day) exhibited liver pathology characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis. There were no apparent effects on the organ weights or organ to body weight ratios. At 500 ppm and 1500/2500 ppm, there were also reported increases in cholesterol, total protein, SGPT and alkaline phosphatase, in addition to a decrease in the albumin/globulin (A/G) ratio throughout the study. Dogs in the 1500/2500 ppm (37.5/62.5 mg/kg/day ai) groups exhibited anorexia, distended abdomens and poor nutritional condition.

The NOAEL is 100 ppm (2.5 mg/kg/day ai). The LOAEL is 500 ppm (12.5 mg/kg day ai) based on biochemical and histological alterations indicating liver damage. Histopathological lesions of the liver were characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis in both sexes of dogs. This study is **acceptable** and satisfies the guideline for a chronic feeding study in dogs (83-1b).

In a more recent study (Accession # 265664, Stadler et al. 1986) beagle dogs (5/sex dose) were administered carbendazim (98.8% ai) at dietary doses levels of 0, 100, 200 or 500 ppm for one year. Based on compound intake, these doses were equivalent to 0, 2.93, 6.43 or 16.54

mg/kg/day in females and 0, 3.2, 7.19 or 17.07 mg/kg/day in males (average for both sexes were 0, 3.06, 6.81 or 16.8 mg/kg/day).

There were no treatment-related effects on clinical observations, body weight, food consumption, hematology, and urinalysis. The only possible treatment-related observation was an increase in cholesterol. Although these values were noted as within the historical control range for the laboratory, actual historical control ranges were not given in the report. In several other dog studies with carbendazim, there were definite dose-related cholesterol increases at higher doses and a borderline increase at 500 ppm. Therefore, it is possible that this change, although minimal and transient, is treatment-related. There was a statistical increase in relative renal weight in the mid and high dose males, however, there were no corresponding effects in clinical chemistries or histopathology. Renal weights were not affect in other carbendazim dog studies. There were slight brain weight changes only in the mid dose group. Therefore, renal and brain weight changes are probably due to individual animal variation. One high dose female had a thyroid follicular adenoma that is considered rare in dogs of this age. However, this tumor was not considered to be treatment-related because there were no corresponding changes in thyroid histology and organ weight or changes in clinical chemistries other than the possible cholesterol increase. This study is **acceptable** and satisfies the guideline for a chronic feeding study in dogs (83-1b).

Classification of Carcinogenic Potential

See previous discussion under benomyl carcinogenic potential classification.

d. Developmental Toxicity

There is increased sensitivity of rat and rabbit fetuses as compared to maternal animals following *in utero* exposure to MBC, in prenatal developmental toxicity studies. In the MBC rat study, increased sensitivity manifested as developmental anomalies (decreased fetal body weight and increases in skeletal variations and a threshold for malformations) at doses which were not maternally toxic. At higher doses, treatment-related malformations of the CNS were observed which included exencephaly, domed head, anophthalmia, microphthalmia and bulged eyes. For developmental toxicity the NOAEL was 10 mg/kg/day, whereas for maternal toxicity, the NOAEL was 20 mg/kg/day (based on a slight increase in liver weight at 90 mg/kg/day).

In the rabbit developmental study with MBC, increased sensitivity manifested as decreased implantations and litter size, and increased resorptions at 20 mg/kg/day; the developmental NOAEL is 10 mg/kg/day. Maternal toxicity was not observed until higher doses of 125 mg/kg/day, based on abortions and decreased maternal body weight; the maternal NOAEL is 20 mg/kg/day.

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